OLIGOPEPTIDIC INHIBITORS OF ELASTASES AND METHODS OF PREPARATION THEREOF

Technical Field

The invention refers to oligopeptidic inhibitors of elastases, especially leukocyte elastase (LE). Elastolytic enzymes, for instance LE or pancreatic elastase (PE) are the members of the group of serine proteinases which take part in the degradation of the natural substrate elastin; elastin creates the substantial part of protein pool of organism. In the healthy organism this destructive activity is inhibited by endogenic enzymes, for instance α_1–proteinase inhibitor (α_1–PI). In the case of insufficiency of α_1–PI of various causes (infection, nutrition, working environment, genetic factors) the autodigestion of organs essential to life by LE and PE is observed, which is the basis of origin of serious diseases as for instance acute pancreatitis, pulmonary emphysema, arthritis or gingivitis.

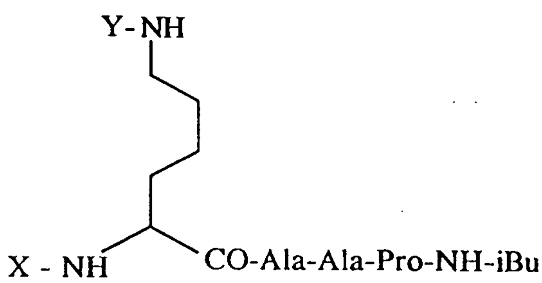
Background Art

One of the possibilities how to reach homeostasis - the balance between elastase and the corresponding endogenic inhibitor (in the case of a pathogenic condition) is the substitution therapy which means the application of the exogenic natural or synthetic inhibitor of type α_1–PI. This is carried out by application of either natural (Antilysin^R, Trasylol^R, Contrykal^R) or synthetic α_1–PI like Nafamstat mesylate, FUT (Tori Co, Tokyo, Japan). Recently, the inventors have suggested a synthetic potent inhibitor for the therapy of acute pancreatitis Glt–Ala_3–NHEt, Inpankin VÚFB 16834 (Gut 33, 701-706, 1992).

The serious defect of the isolated (natural) inhibitors is not only their antigenicity (α_1–PI shows the species specificity), but also the possible contamination by bovine spongioformic encephalopathy (BSE). On the contrary, the synthetic, low molecular inhibitors are lacking these unwanted side effects, are more efficient and more stable. Their chemical structure is strictly defined and characterised in the chemical and physical way.

Disclosure of Invention

The invention relates to oligopeptidic inhibitors of elastases of general structure I.



(I),

wherein

X means A or B, wherein A is different from B

Y means A or B or A–Ala, wherein A is different from B and if Y is A–Ala then X means B,

A means a saturated acid of 8 to 16 carbon atoms,

B means the 3–carboxypropionyl or 4–carboxybutyroyl residue.

According to a further aspect of the present invention there is provided a method for the preparation of compounds of general structure I wherein Y is B, characterised in contacting lysine having protected amino groups with a compound of formula

Ala-Ala-Pro-NH-iBu,

deprotecting the ε-amino group of the product, reacting the resulting product with a compound of formula

$A\text{-}X^1$,

wherein A has the above meaning and X^1 is a halogen, deprotecting the α-amino group and contacting the resulting compound of formula Lys(A)-Ala-Ala-Pro-NH-iBu with succinic or glutaric anhydride.

The invention also includes a method for the preparation of compounds of general structure I wherein Y means A, characterised in contacting a compound of formula A-Lys(W), wherein W is a protective group and A has the above meaning, with a compound of formula

Lys(W), wherein W is a protective group and A has the above meaning, with a compound of formula

Ala-Ala-Pro-NH-iBu,

deprotecting the product and contacting the resulting compound of formula A-Lys-Ala-Ala-Pro-NH-iBu with succinic or glutaric anhydride.

The invention also includes the method for the preparation of compounds of general structure I wherein Y is A-Ala, characterised in contacting a compound of formula A-Ala with a compound of formula

W-Lys-Ala-Ala-Pro-NH-iBu,

wherein W is a protective group and A has the above meaning, deprotecting the product and contacting the resulting compound of formula Lys(A-Ala)-Ala-Ala-Pro-NH-iBu with succinic or glutaric anhydride.

Compounds of general structure I are efficient competitive inhibitors of serine proteinases, especially of leukocyte elastase (LE) and pancreatic elastase (PE). These enzymes are the cause of destructive damage of cell and venous walls and therefore are the cause of serious diseases. Besides the mostly observed pulmonary emphysema, acute pancreatitis and various types of arthritis, the above mentioned enzymes cause hemorrhagic periodontitis. This is the reason why one of the attractive applications of the compounds described is the use of these as the ingredients of various dental preparations, for instance dentifrices or mouth washes. An especially interesting one would be the application of these efficient inhibitors as ingredients of chewing gums; the ingredients in these medicinal preparations ensure the constant level of the active inhibitor in oral cavity also during the daily working activity, when, from the working and time reasons the standard hygienic care of the dental cavity is not possible.

Further potential use of LE inhibitors is in blocking of the activation of pro-enzymes, for instance cathepsine B (Biochim.Biophys. Acta 1226, 117-125, 1994) or for inhibition of matrix metalloproteinase 3 (MMP-3) -so called stromelysin from the corresponding zymogen (FEBS Lett. 299, 353-356, 1989). MMP-3 is known as the key enzyme in pathogenesis of various malign diseases and also in sclerosis multiplex.

The guarantee of the lack of the unwanted or toxic side effect of the inhibitors described is the fact that these are synthesised only from physiologic, so nontoxic compounds, which means aminoacids (alanine, proline, lysine), higher fatty acids and succinic or glutaric acids. Compounds of general structure I can be used in the form of free acids or (for the reason of higher solubility) in the form of soluble salts created with alkali metals, mostly with sodium. The synthesis of oligopeptidic inhibitors of elastase according to this invention including all the intermediates is described in schemes 1, 2 and 3. The final products and intermediates are characterized by their melting points (m.p.) or by their optical rotations, in some cases by thin layer chromatography (TLC). The values are mentioned in tables I-VI.

The key compound for synthesis of all oligopeptidic inhibitors is isobutylamide of alanyl–alanyl–proline (Collection Czechoslov. Chem. Commun. 52, 3034-3041, 1987); acylation of aminoacids by higher fatty residues is described in patent document No CS 280 726. The synthesis of intermediates and of final products is performed in solution and is clear from the examples of preparation. The evaluation (determination of inhibitory constants Ki) by human leukocyte elastase was performed using known procedure (Biol. Chem. Hoppe-Seyler 366, 333-343, 1985), the results of determinations of some compounds are described in table VII.

Following abbreviations and symbols were used:

Ala = alanine

Pro = proline

Lys = lysine

Suc = succinyl

Glt = glutaryl

Kpl = caproyl

Lau = lauroyl

Myr = myristoyl

Pal = palmitoyl

Z = benzyloxycarbonyl

Boc = tert.butyloxycarbonyl

iBu = isobutyl

DCCI = N,N'-dicyclohexylcarbodiimide
OHSuc = N–hydroxysuccinimide
EtO-CO-Cl = ethyl chloroformate
Suc-O = succinic anhydride

The standard preparation: the rough reaction product was solubilized in AcOEt and extracted with 1% citric acid, followed by extraction with H_2O, then with 5% $NaHCO_3$ and again with H_2O. The product was dried over anhydrous Na_2SO_4 and evaporated. All the evaporation was done under reduced pressure on a rotary vacuum evaporator.

Thin layer chromatography (TLC - silica-gel G) was performed in the system S_1: n–butanol–acetic acid–H_2O (4:1:1).

Optical rotation was determined using the Perkin-Elmer 141 polarimeter. Melting points were determined on the Koffler block and have not been corrected.

Scheme 1

Boc-Lys(Z) + Ala-Ala-Pro-NH-iBu
↓
Boc-Lys(Z)-Ala-Ala-Pro-NH-iBu
↓ H_2/Pd
Boc-Lys-Ala-Ala-Pro-NH-iBu
↓ Pal-Cl
Boc-Lys(Pal)-Ala-Ala-Pro-NH-iBu
↓ TFA/anex L 150
Lys(Pal)-Ala-Ala-Pro-NH-iBu
↓ Suc-O
Suc-Lys(Pal)-Ala-Ala-Pro-NH-iBu
I

Scheme 2

A-Lys(Z)-Ala-Ala-Pro-NH-iBu
↓ H_2/Pd II(a-d)
A-Lys-Ala-Ala-Pro-NH-iBu
↓ B - O III(a-d)
A-Lys(B)-Ala-Ala-Pro-NH-iBu
IV(a-h)

II, III	A
a	Kpl
b	Lau
c	Myr
d	Pal

IV	A	B
a	Kpl	Suc
b	Lau	Suc
c	Myr	Suc
d	Pal	Suc
e	Kpl	Glt
f	Lau	Glt
g	Myr	Glt
h	Pal	Glt

Scheme 3

Boc–Lys–Ala–Ala–Pro–NH–iBu
↓
Boc–Lys(Y)–Ala–Ala–Pro–NH–iBu

V(a-d) ↓ 1) HCl/AcOH 2) anex (Zerolit)
Lys(Y)–Ala–Ala–Pro–NH–iBu

VI(a-d) ↓ Suc–O
Suc–Lys(Y)–Ala–Ala–Pro–NH–iBu
VII(a-d)

V, VI, VII	Y
a	Kpl–Ala
b	Lau–Ala
c	Myr–Ala
d	Pal–Ala

Table I

II (a-d)		M.p. °C
a	Kpl-Lys(Z)-Ala-Ala-Pro-NH-iBu	205-208
b	Lau-Lys(Z)-Ala-Ala-Pro-NH-iBu	188-190
c	Myr-Lys(Z)-Ala-Ala-Pro-NH-iBu	178-181
d	Pal-Lys(Z)-Ala-Ala-Pro-NH-iBu	169-172

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Table II

III (a-d)		M.p. °C
a	Kpl-Lys-Ala-Ala-Pro-NH-iBu	177-181
b	Lau-Lys-Ala-Ala-Pro-NH-iBu	183-185
c	Myr-Lys-Ala-Ala-Pro-NH-iBu	173-176
d	Pal-Lys-Ala-Ala-Pro-NH-iBu	154-158

Table III

IV (a-h)		M.p. °C
a	Kpl-Lys(Suc)-Ala-Ala-Pro-NH-iBu	141-144
b	Lau-Lys(Suc)-Ala-Ala-Pro-NH-iBu	143-145
c	Myr-Lys(Suc)-Ala-Ala-Pro-NH-iBu	148-151
d	Pal-Lys(Suc)-Ala-Ala-Pro-NH-iBu	142-144
e	Kpl-Lys(Glt)-Ala-Ala-Pro-NH-iBu	126-128
f	Lau-Lys(Glt)-Ala-Ala-Pro-NH-iBu	152-155
g	Myr-Lys(Glt)-Ala-Ala-Pro-NH-iBu	147-150
h	Pal-Lys(Glt)-Ala-Ala-Pro-NH-iBu	124-126

Table IV

V (a-d)		M.p. °C
a	Boc-Lys(Kpl-Ala)-Ala-Ala-Pro-NH-iBu	179-182
b	Boc-Lys(Lau-Ala)-Ala-Ala-Pro-NH-iBu	181-184
c	Boc-Lys(Myr-Ala)-Ala-Ala-Pro-NH-iBu	133-135
d	Boc-Lys(Pal-Ala)-Ala-Ala-Pro-NH-iBu	146-150

Table V

VI (a-d)		M.p. °C
a	Lys(Kpl-Ala)-Ala-Ala-Pro-NH-iBu	185-188
b	Lys(Lau-Ala)-Ala-Ala-Pro-NH-iBu	189-191
c	Lys(Myr-Ala)-Ala-Ala-Pro-NH-iBu	184-187
d	Lys(Pal-Ala)-Ala-Ala-Pro-NH-iBu	158-161

Table VI

VII (a-d)		M.p. °C
a	Suc-Lys(Kpl-Ala)-Ala-Ala-Pro-NH-iBu	182-186
b	Suc-Lys(Lau-Ala)-Ala-Ala-Pro-NH-iBu	188-189
c	Suc-Lys(Myr-Ala)-Ala-Ala-Pro-NH-iBu	181-184
d	Suc-Lys(Pal-Ala)-Ala-Ala-Pro-NH-iBu	171-174

Table VII

Inhibitory constants (Ki) of human leukocyte elastase		
IIIa	Kpl-Lys-Ala-Ala-Pro-NH-iBu	$2{,}27.10^{-5}$ M
IVa	Kpl-Lys(Suc)-Ala-Ala-Pro-NH-iBu	$4{,}50.10^{-6}$ M
IVb	Lau-Lys(Suc)-Ala-Ala-Pro-NH-iBu	$5{,}32.10^{-6}$ M
IVc	Myr-Lys(Suc)-Ala-Ala-Pro-NH-iBu	$6{,}82.10^{-6}$ M
IVe	Kpl-Lys(Glt)-Ala-Ala-Pro-NH-iBu	$9{,}96.10^{-6}$ M
IVf	Lau-Lys(Glt)-Ala-Ala-Pro-NH-iBu	$7{,}35.10^{-6}$ M
IVh	Pal-Lys(Glt)-Ala-Ala-Pro-NH-iBu	$1{,}46.10^{-5}$ M
VIb	Lys(Lau-Ala)-Ala-Ala-Pro-NH-iBu	$5{,}38.10^{-7}$ M
VIIb	Suc-Lys(Lau-Ala)-Ala-Ala-Pro-NH-iBu	$6{,}92.10^{-7}$ M
VId	Lys(Pal-Ala)-Ala-Ala-Pro-NH-iBu	$2{,}47.10^{-5}$ M
VIId	Suc-Lys(Pal-Ala)-Ala-Ala-Pro-NH-iBu	$1{,}19.10^{-5}$ M

<u>Examples</u>

Example 1

Boc-Lys(Z)-Ala-Ala-Pro-NH-iBu

To the solution of Boc-Lys(Z) (20 mmol) and N-Etp (2,8 ml) in DMFA (50 ml) cooled to -10 °C EtOCO-Cl (2 ml) was added; after 5 min stirring (-10 °C) to the reaction mixture the solution of Ala-Ala-Pro-NH-iBu (6,3 g, 20 mmol) in 20 ml DMFA was added. The mixture was stirred for 30 min (0 °C) and then the stirring continued for 2 h (room temperature). After this, the reaction mixture was evaporated, the residue solubilized in the mixture of AcOEt and H_2O and standard procedure I followed. The residue was crystallised from 2–propanol and light petroleum. 5,7 g of the product (m.p. 168-170 °C) were obtained.

Boc-Lys-Ala-Ala-Pro-NH-iBu

To the solution of Boc-Lys(Z)-Ala-Ala-Pro-NH-iBu (3,4 g; 5 mmol) in methanol (300 ml) the suspension 5% Pd/C (0,3 g) in toluene (10 ml) was added and the reaction mixture was hydrogenated in autoclave 20 min, under the pressure 2 MPa. After removing of the catalyst by filtration, the methanol solution was evaporated. 2,8 g of product (m.p. 150-153 °C) was obtained.

Boc-Lys(Pal)-Ala-Ala-Pro-NH-iBu

To the solution of Boc-Lys-Ala-Ala-Pro-NH-iBu (1,1 g, 2 mmol) in DMFA (4 ml) and 1 M NaOH (3,5 ml) Pal-Cl (1 ml) during 30 min was added (room temperature); after further 30 min of stirring the reaction mixture was acidified (10% AcOH, pH 5) and the crude precipitate formed was separated by filtration. 0,9 g of product (chromatographic homogeneity, $R_f = 0,71/S_1$) was obtained.

Lys(Pal)-Ala-Ala-Pro-NH-iBu

To the solution of Boc-Lys(Z)-Ala-Ala-Pro-NH-iBu (390 mg) in AcOH (0,5 ml) TFA (0,5 ml) was added. After 1 h, when the mixture was standing at the room temperature it was evaporated and dried over P_2O_5. Then, the trifluoracetate obtained was solubilized in

methanol and deionised using slightly basic anex (L 150). 180 mg of product (base, m.p. 139-142 °C; $[\alpha]^{20}_D$ -78,3° (c = 0,2; methanol) was obtained.

Suc-Lys(Pal)-Ala-Ala-Pro-NH-iBu

To the solution of Lys(Pal)-Ala-Ala-Pro-NH-iBu (140 mg, 0,2 mmol) in DMFA (20 ml) succinic anhydride (25 mg) was added; the reaction mixture was kept at the temperature 80 °C for 30 min, then the reaction mixture was evaporated and gel residue was crystallised from the solution of DMFA and AcOEt. 60 mg of product (m.p. 157-160 °C; $[\alpha]^{20}_D$ -73,5° /c = 0,2; methanol/) was obtained.

Example 2

Myr-Lys(Z)-Ala-Ala-Pro-NH-iBu (IIc)

To the solution of Myr-Lys(Z) (5,05 g, 10 mmol) in DMFA (150 ml), HOSuc (1,15 g) in DMFA (20 ml) was added after cooling to 0 °C DCCI (2,2 g) was added. After 1 h stirring (0 °C) Ala-Ala-Pro-NH-iBu (3,15 g, 10 mmol) in DMFA (25 ml) was added. The reaction mixture was left to stand for 12 h at room temperature, then AcOH (0,2 ml) was added and the reaction suspension was left at 0 °C for 30 min. Then it was filtered, washed by DMFA and evaporated. The gel residue was crystallised from hot DMFA (40 ml). 4,7 g of product (m.p. 178-181 °C) was obtained. The similar procedure was used for the preparation of compounds II (a,b,d).

Myr-Lys-Ala-Ala-Pro-NH-iBu (IIIc)

To the solution of Myr-Lys(Z)-Ala-Ala-Pro-NH-iBu (4 g; 5 mmol) in methanol (300 ml), the suspension of 5% Pd/C (0,3 g) was added the pressure hydrogenolysis was performed (2 MPa) as described in example 1. 2,9 g of product (m.p. 173-176 °C) was obtained. The similar procedure was used for the preparation of compounds III (a,b,d).

Myr-Lys(Suc)-Ala-Ala-Pro-NH-iNH-iBu (IVc)

To the solution of Myr-Lys-Ala-Ala-Pro-NH-iBu (1,35 g, 2 mmol) in dioxane (30 ml) succinic anhydride (380 mg) was added and the reaction mixture was heated for 30 min

under reflux. After cooling, 1,75 g of product (m.p. 148-151 °C) was obtained. Similar procedure was used for preparation of compounds IV (a,b,d).

Pal-Lys(Glt)-Ala-Ala-Pro-NH-iBu (IVh)
To the solution of Pal-Lys-Ala-Ala-Pro-NH-iBu (2.05 g, 3 mmol) in dioxane (30 ml) glutaric anhydride (600 mg) was added and the reaction mixture was heated for 30 min under the reflux. After cooling 2,1 g of product was obtained (m.p. 124-126 °C; $[\alpha]^{20}_{D}$ -34,6° /c = 0,2; DMFA/). Similar procedure was used for the preparation of compounds IV (e-g).

Example 3

Boc-Lys(Pal-Ala)-Ala-Ala-Pro-NH-iBu (Vd)
To the solution Pal-Ala (985 mg, 3 mmol) in DMFA (25 ml) OHSuc (350 mg) was added, after cooling to -5 °C DCCI (660 mg) was added, after 30 min of stirring (-5 °C) the solution of Boc-Lys-Ala-Ala-Pro-NH-iBu (1,65 g, 3 mmol) in DMFA (30 ml) was added. The mixture was stirred at room temperature for 3 h. The created precipitate was separated by filtration, washed DMFA and water (50 ml) was added. The precipitate was separated by filtration and crystallised from 2–propanol. 1,75 g of product (m.p. 146-150 °C) was obtained. The similar procedure was used for the preparation of compounds V(a-c).

Lys(Pal-Ala)-Ala-Ala-Pro-NH-iBu (IVd)
To the solution of Boc-Lys(Pal-Ala)-Ala-Ala-Pro NH-iBu (1,7 g; 2 mmol) in AcOH (20 ml) the solution of 0,6 M HCl/AcOH (4 ml) was added. After 3 h of standing at room temperature, the created hydrochloride was precipitated by means of ether, separated by filtration and dried in the presence of P_2O_5 and NaOH. Then, the hydrochloride was deionised by strong basic anex (Zerolit FF/OH-cycle) in methanol. The solution was evaporated and the residue was crystallised from 2–propanol and light petroleum. The base (820 mg) was obtained (m.p. 158-161 °C). The similar procedure was used for the preparation of compounds VI(a-c).

Suc-Lys(Pal-Ala)-Ala-Ala-Pro-NH-iBu (VIId)

To the solution of Lys(Pal-Ala)-Ala-Ala-Pro-NH-iBu (750 mg; 1 mmol) in DMFA (20 ml) succinic anhydride (160 mg) was added. After 2 h of stirring at the room temperature the product was precipitated by addition of H_2O (50 ml) and crystallised from 2–propanol and AcOEt. 690 mg of the final product was obtained (m.p. 171-174 °C). The similar procedure was used for the preparation of compounds VII(a-c).